ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Thirty-second Quarterly Report of Progress

Order No. W-13411

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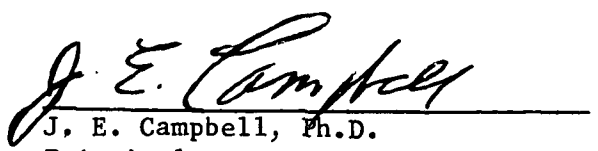

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Introduction

The previous report showed that survivor curves of Bacillus subtilis var. niger spores heated at 113°C under 1.3 μg and 0.001 μg of water per cm^3 were non-log linear in the range of mean concentrations of 10^6 to approximately 10^{-3} organisms per test.

For the past several years, dry heat inactivation studies of B. subtilis var. niger spores were conducted under conditions of low relative humidity (RH). However, during recent studies under conditions of near 100% RH or in aqueous solution, a few organisms not typical of the B. subtilis var. niger colonies were observed. The question was raised whether the presence of these atypical organisms have an effect on the nature of the survivor curve under conditions of low relative humidity.

In view of these findings, efforts were concentrated on (1) determining the heat sensitivity of these atypical, white spores treated under dry heat conditions and their influence on the nature of the survivor curve; (2) preparation of a new spore crop obtained from spore isolates by a purification procedure; and (3) comparing spore crops obtained from Cape Kenedy (SSM-10), Minnesota (Minn. sp. AAEF), "old" Cincinnati spore crop, and the new purified Cincinnati spore crop under dry heat conditions.

I. EXPERIMENTAL

A. Dry heat studies

The experiment was carried out in the conventional way. Two sets of spore crops of the same species and a variety of species from Cape Kennedy (SSM-10) and Minnesota (Minn. sp. AEFF) were obtained in order to compare their heat resistance under 0.134% RH (25 STP) at 113°C with the "old" spore crop and "new" purified spore crops. Spores were assayed in the range of mean concentrations of 10^6 to approximately 10^{-3} organisms per test unit. The spores were prepared in a double-distilled water suspension (for plate counts) and in a 95%-ethanol suspension (for MPN estimates), and were dispensed with a repeating dispenser in 0.01 ml amounts in stainless steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 206 mm x 300 mm tin cans. Each of the four shelves contained 30 cups for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 100 minutes at 45° to 50°C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 minutes for the first 100 minutes, followed by five consecutive purges of nitrogen with a vacuum cycle between each purge.

After drying the cans, lids, and contents were removed from the oven and cooled to about 30°C in the equilibration hood. The cans were sealed, removed from the equilibration hood, and soldered around the seams to prevent any water vapor loss during the heating cycle. Heat treatment was applied at regular time

intervals, and the cans were cooled immediately in a 40°F recirculating water bath.

Spore survivors from 10^6 to 10^0 spores per cup were assayed by sonifying the cups in peptone water, plating, and counting in TGE agar.

When spore survivors ranged from < 1 to 10^{-3} spores per test unit, the most probable number method was used. TGE broth in 0.5 ml amounts was placed in each cup; the cups were incubated for 7 days at 35°C, and were scored for growth or nongrowth.

B. Purification study

1. Selection of isolates

It was decided that a single cell from an isolated colony could be recovered and the density of the cell estimated by the most probable number (MPN) procedure.

Typical and atypical isolates were chosen from streaked plates obtained from samples treated under wet heat and also from samples taken from the original spore stock suspension.

2. MPN procedure

Dilution for MPN determinations was made in 99-ml blanks of phosphate buffered water. The initial dilution was made from an isolated growing colony on a TGE agar plate. Tenfold serial dilutions were made, and 1-ml each from three decimal dilutions were transferred into a series of 10 tubes each containing 5 ml

of TGE broth. This series represented an MPN level of approximately 10^0, 10^{-1}, and 10^{-2} cells per ml. The tubes were shaken and incubated at 35°C for a minimum of 3 days. These isolation and MPN procedures were repeated 5 times to produce a statistical chance of 1 in 3.5×10^6 of obtaining a non-pure culture.

New spore crops were produced from the isolates and were harvested under the conventional procedure.

Results

A comparison of thermal inactivation curves for B. subtilis var. niger spores under 0.134% RH (STP) is presented in Figures 1, 2, 3, and 4. In all cases, by visual examination, survivor curves appear essentially the same in the mean concentration range of 10^6 to 10^0 spore survivors per cup.

The sensitivity of the test was further extended in the mean concentration range of < 1 to 10^{-3} spore survivors per test unit by the most probable number method. These data are also shown in Figures 1, 2, 3, and 4. The survivor curves appear the same up to 18 hours on all four spore crops tested. Results after 18 hours were observed to be negative for 9 of 12 points.

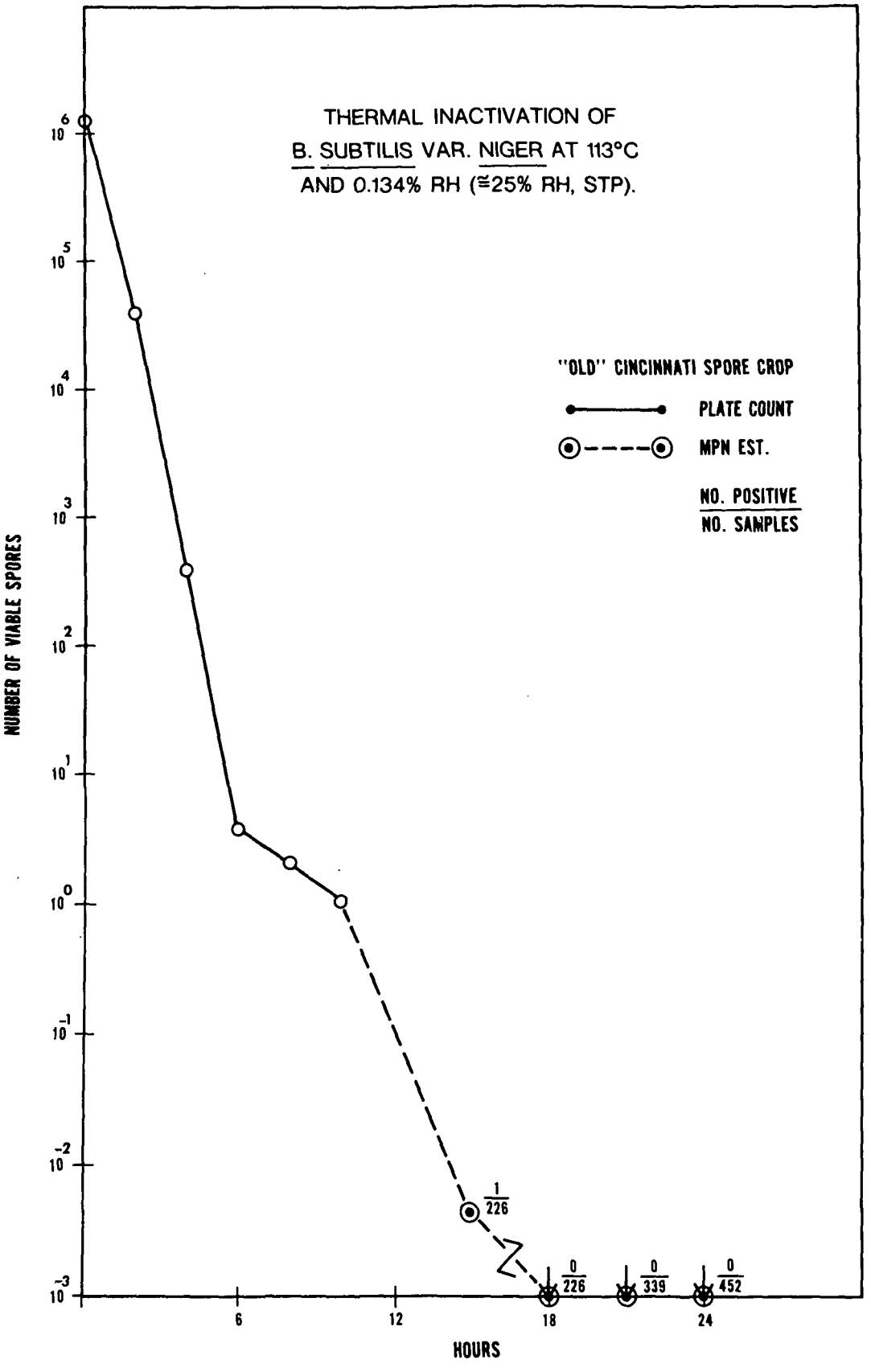
THERMAL INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C
AND 0.134% RH (≅25% RH, STP).
"OLD" CINCINNATI SPORE CROP
PLATE COUNT
MPN EST.
NO. POSITIVE
NO. SAMPLES
NUMBER OF VIABLE SPORES
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
1/226
0/226
0/339
0/452
6
12
18
24
HOURS

FIGURE 1

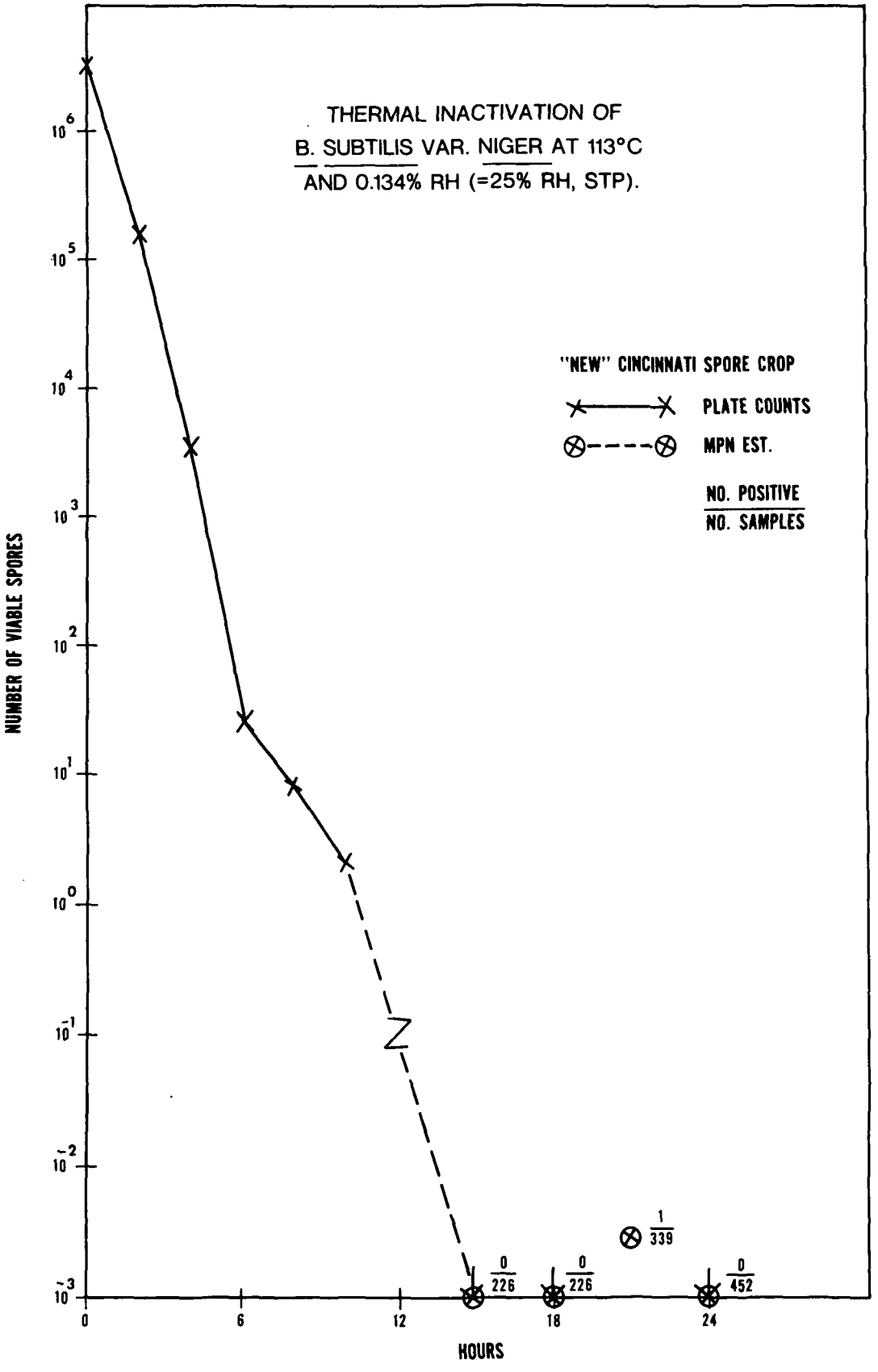
THERMAL INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C
AND 0.134% RH (=25% RH, STP).
"NEW" CINCINNATI SPORE CROP
PLATE COUNTS
MPN EST.
NO. POSITIVE
NO. SAMPLES
NUMBER OF VIABLE SPORES
HOURS
0
6
12
18
24
0/226
0/226
1/339
0/452

FIGURE 2

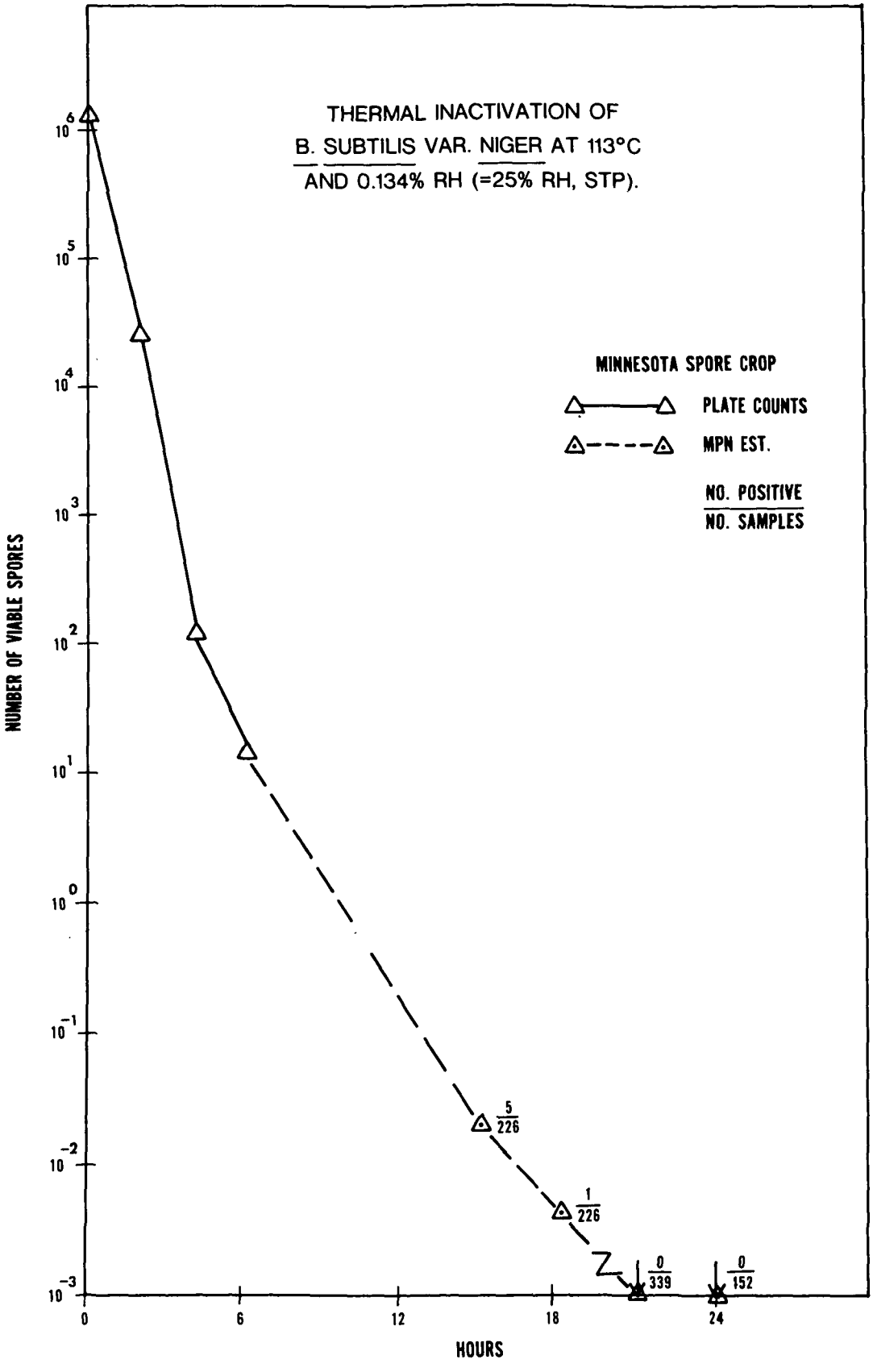
THERMAL INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C
AND 0.134% RH (=25% RH, STP).
MINNESOTA SPORE CROP
PLATE COUNTS
MPN EST.
NO. POSITIVE
NO. SAMPLES
NUMBER OF VIABLE SPORES
HOURS
5/226
1/226
0/339
0/152

FIGURE 3

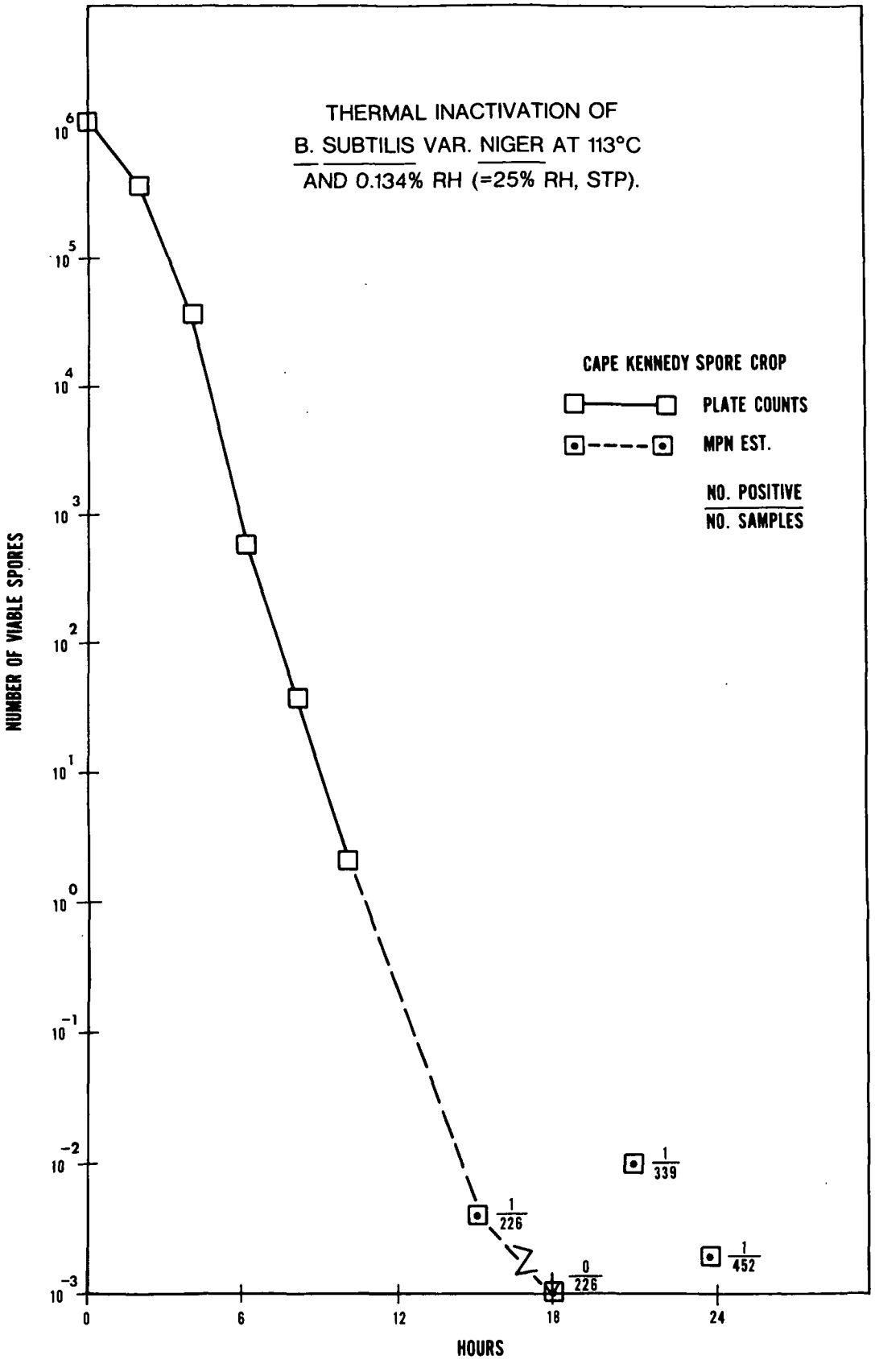
THERMAL INACTIVATION OF
B. SUBTILIS VAR. NIGER AT 113°C
AND 0.134% RH (=25% RH, STP).
CAPE KENNEDY SPORE CROP
PLATE COUNTS
MPN EST.
NO. POSITIVE
NO. SAMPLES
NUMBER OF VIABLE SPORES
HOURS
1/339
1/226
1/452
0/226

FIGURE 4